PATENT COOPERATION TREATY

PCT

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

28 JUN 2005

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference UMD-0019	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US03/41136	International filing date *(day/month/year)* 24 December 2003 (24.12.2003)	Priority date *(day/month/year)* 27 December 2002 (27.12.2002)
International Patent Classification (IPC) or national classification and IPC IPC(7): C12Q 1/68; C12P 19/34; C07H 21/02, 21/04 and US Cl.: 435/6, 91.1, 91.2; 536/23.1, 24.3, 24.31		
Applicant UNIVERSITY OF MEDICINE AND DENTISTRY OF NEW JERSEY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of report with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20 July 2004 (20.07.2004)	14 February 2005 (14.02.2005)
Name and mailing address of the IPEA/US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Dorothea Lawrence for Carla Myers Telephone No. 571-272-1600

Form PCT/IPEA/409 (cover sheet)(July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International applica[redacted]No.
PCT/US03/41136

I. Basis of the report

1. With regard to the elements of the international application:*

☐ the international application as originally filed.

☒ the description:
pages 1-20 as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the claims:
pages NONE, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages 21 and 22, filed with the letter of 15 November 2004 (15.11.2004)

☒ the drawings:
pages 1-3, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the sequence listing part of the description:
pages 1-25, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in printed form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages NONE

☐ the claims, Nos. NONE

☐ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US03/41136

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-11	YES
	Claims NONE	NO
Inventive Step (IS)	Claims NONE	YES
	Claims 1-11	NO
Industrial Applicability (IA)	Claims 1-11	YES
	Claims NONE	NO

2. CITATIONS AND EXPLANATIONS
Please See Continuation Sheet

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

V. 2. Citations and Explanations:

Claims 1, 2, 4-6, and 8-10 lack an inventive step under PCT Article 33(3) as obvious over Nazarenko in view of Metallinos and Matsuzaki. Nazarenko (see column 25) teaches a method for detecting the presence of a single nucleotide polymorphism or a mutation in a target nucleic acid wherein the method comprises: (i) amplifying a nucleic acid sequence using a hairpin primer, wherein the primer terminates at a polymorphic position; and (ii) measuring the amount of amplification product wherein a decrease in the amplification product is indicative of the presence of a polymorphism or mutation. Nazarenko (column 25) teaches that in the method of allele specific PCR, "(u)nder the appropriate reaction conditions, the target DNA is not amplified if there is a base mismatch." With respect to claims 5 and 6, Nazarenko (column 16) teaches that the hairpin primer may be DNA or RNA. With respect to claims 8-10, Nazarenk0 (column 32) teaches kits comprising the reagents necessary to perform allele specific PCR wherein the kits comprise a hairpin primer that terminates at its 3' end at the location of a single nucleotide polymorphism or mutation. Nazarenko is silent with respect to the length of the amplification products synthesized by the polymerase chain reaction. However, Matsuzaki teaches that PCR is more efficient when smaller length nucleic acids are amplified. The reference (column 1) states that "The yield of longer amplicons is often less than the yield of shorter amplicons because of those differences in PCR amplification efficiency." Further, Metallinos (column 11) exemplifies methods of allele specific PCR in which the amplification products are of a length of 90 bp. In view of the teachings of Matsuzaki and Metallinos, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have practiced the method of Nazarenko so that PCR primers were selected to yield products of a smaller length, e.g., products of a length of 90 nucleotides or less, in order to have improved the efficiency of PCR and to have increased the yield of the target amplification product.

Claims 3, 7 and 11 lack an inventive step under PCT Article 33(3) as obvious over Nazarenko in view of Metallinos and Matsuzaki and further in view of Tyagi. The teachings of Nazarenko, Metallinos and Matsuzaki are presented above. With respect to claim 3, Nazarenko teaches detecting PCR amplification products at the completion of the PCR assay. Nazarenko does not teach detecting PCR products using real-time PCR. However, Tyagi teaches a method of allele specific PCR (column 3) wherein amplification products are measured either in real-time or at the end-point of the assay (column 4). Tyagi teaches that the primer used for PCR may be a hairpin primer (column 6). Tyagi (column 2) also teaches that "if the binding of the primer in the tube to the target sequence creates a mismatched 3'-terminal nucleotide, then the primer cannot be efficiently extended by incubation with DNA polymerase. Amplification of the mismatched template is significantly delayed." In view of the teachings of Tyagi, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Nazarenko so as to have detected the amplification products in real-time, rather than at the completion of PCR, because Tyagi teaches that real-time PCR provides an equally effective means for monitoring allele-specific amplification.

With respect to claims 7 and 11, Nazarenko does not teach performing allele-specific PCR using hairpin primers that contain PNAs. However, Tyagi (column 6) teaches that hairpin primers used for allele specific PCR may contain PNAs. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Nazarenko so as to

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

have performed the allele-specific PCR method using hairpin primers that contain PNAs in view of the well known benefits provided by PNAs of enhancing the stability of hybridization and improving the ability to distinguish between perfectly matched and mismatched sequences. Thereby, one would have been motivated to have used PNA hairpin primers in order to have provided a more sensitive and effective method for detecting the presence of a polymorphism or mutation.

Claims 1-11 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry for detecting the presence of a mutation or polymorphism in a target nucleic acid.

In the response filed November 15, 2004, the previous rejection was traversed. The response states that the claims have been amended to recite that the amplification product is of a length of 30 to 90 nucleotides, and that the cited prior art does not teach this embodiment. However, as set forth in the above rejection, the concept of producing smaller amplification products was known in the art as exemplified by the teachings of Metallinos and Matsuzaki. In view of the teachings of Metallinos and Matsuzaki, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have practiced the method of Nazarenko using PCR primers that yield smaller amplification products of a length of about 90 nucleotides or length in order to have increased the efficiency of PCR.

---------- NEW CITATIONS ----------

US 6,333,179 B1 (MATSUZAKI et al) 25 December 2001 (25.12.2001), see column 1.
US 6,372,900 B1 (METALLINOS et al) 04 April 2002 (16.04.2002), see column 11.

What is claimed is:

Claim 1: An assay for detecting a single nucleotide polymorphism in an organism comprising:

amplifying a 30 to 90 base pair nucleic acid molecule of an organism using a hairpin shaped primer that discriminates between different alleles by situating its 3′ nucleotide at the location of a single nucleotide polymorphism; and

measuring threshold cycle or amplification efficiency or amount of amplified product wherein a lower amplification efficiency or delayed threshold cycle or a difference in the amount of amplified product is indicative of a mismatch between the primer and the organism and a single nucleotide polymorphism in the organism.

Claim 2: The assay of claim 1 wherein the nucleic acid sequence of the organism is amplified by PCR.

Claim 3: The assay of claim 2 wherein the PCR performed is real-time PCR.

Claim 4: The assay of claim 2 wherein amplicon production is measured at the completion of the PCR reaction.

Claim 5: The assay of claim 1 wherein the hairpin shaped primer comprises DNA.

Claim 6. The assay of claim 1 wherein the hairpin shaped primer comprises RNA.

Claim 7: The assay of claim 1 wherein the hairpin shaped primer comprises PNA.

Claim 8: An assay kit for detecting a single nucleotide polymorphism in an organism comprising a hairpin shaped primer for amplifying a 30 to 90 base pair nucleic acid molecule, wherein the hairpin shaped primer discriminates between different alleles by situating its 3′

nucleotide at the location of a single nucleotide polymorphism.

Claim 9: The assay kit of claim 8 wherein the hairpin shaped primer comprises DNA.

Claim 10: The assay kit of claim 8 wherein the hairpin shaped primer comprises RNA.

Claim 11: The assay kit of claim 8 wherein the hairpin shaped primer comprises PNA.